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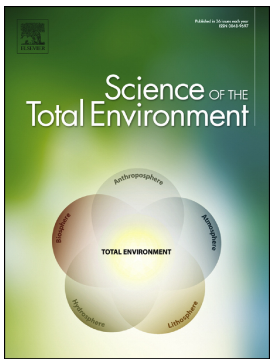

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Edward J. Calabrese, Evgenios Agathokleous

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POLLEN BIOLOGY AND HORMESIS: Pollen Germination and Pollen Tube Elongation

Edward J. Calabrese, Ph.D.*

Professor of Toxicology

Environmental Health Sciences

Morrill I, N344

University of Massachusetts

Amherst MA 01007

Phone: 413-545-3164

Email: edwardc@schoolph.umass.edu

Evgenios Agathokleous

Key Laboratory of Agrometeorology of Jiangsu Province

Institute of Ecology

School of Applied Meteorology

Nanjing University of Information Science & Technology

Nanjing 210044, China.

E-mail: evgenios@nuist.edu.cn

ORCID: 0000-0002-0058-4857

*Corresponding Author

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ABSTRACT

This paper evaluated the occurrence of hormetic dose responses in pollen reported over the past eight decades. Hormetic doses responses were induced by a wide range of chemical and physical agents in 34 plant species for pollen germination and pollen tube growth/elongation. Agents inducing such hormetic dose/concentration responses in pollen included nutrients, growth-promoting agents, plant and animal hormones, toxic substances, including heavy metals such as cadmium, gaseous pollutants such as ozone, as well as ionizing and non-ionizing radiation. This paper provides further evidence for the broad generality of the hormesis dose response, supporting substantial prior findings that the hormetic response is independent of biological model, inducing agent, and endpoints measured. Given the widespread potential of inducing hormetic dose responses in pollen, these findings indicate the need to explore their emerging biological, ecological, agricultural, economic and public health implications.

KEY WORDS: Biphasic dose-response; hormesis; pollen germination; pollen tube elongation; reproduction; stress biology

1. INTRODUCTION

In flowering plants, the reproduction process is mediated by the pollen tube. This tubular structure provides the vehicle by which two immobile sperm cells are transported to the ovule, the location of two female reproduction cells (Sprunck, 2010; Dresselhaus and Franklin-Tong, 2013; Vogler et al., 2014). The pollen tube develops only after the pollen grain is transported, attaches, and then rehydrates on the receptive papillae that reside on the top of the stigma. After this process, the pollen grain becomes activated to form the so-called pollen tube, which may be characterized as a tubular protrusion that is chemically directed via complex physical and sensory processes through the pistil, eventually reaching the female gametophyte. At this point in the process, the male reproductive cells finally arrive at the target destination and initiate the process of fertilization (Palanivelu and Tsukamoto, 2012). Beyond their essential role in fertilization, pollen tubes are widely used to assess biological polarity, with broad applications to other cell types and biological processes.

Of considerable theoretical and practical significance is that the pollen grain displays a self-organizing system that permits pollen to germinate, producing pollen tubes *in vitro* within an external signal mediated framework. The pollen germination rates, pollen tube growth rates, and final tube length are typically lower in *in vitro* studies than those observed in whole plants, most likely due to the absence of female factors that affect pollen germination, tube growth and growth directionality (Johnson and Presse, 2002; Chae and Lord, 2011). The selection of plants for pollen studies is affected by many species-specific processes as there are many factors that affect pollen tube growth, including the morphology of the reproductive tract (e.g. the stigma, style, the septum epidermis, and the funiculus). The nutrient containing extracellular matrix (ECM) contains a nutrient mixture that is important in enhancing pollen tube elongation

(Johnson and Presse, 2002; Chae and Lord, 2011). However, many studies employ bi-cellular pollen from species such as tobacco, which yields reliable findings (Volger et al., 2014).

Considerable research has been directed toward assessing abiotic factors that may affect pollen generation, pollen elongation, and directionality, including inorganic ions, small organic molecules, proteins, polyamines, plant hormones, such as auxin, gibberellic acid, abscisic acid, and bassinosteroids, ionizing and non-ionizing radiation, chemical toxins, such as toxic metals, chemical forms of acid rain, as well as smoke-water extract materials. In fact, an evolutionary based hypothesis that the effect of smoke-water on pollen germination and pollen tube elongation demonstrated biphasic dose responses (Paperfus et al., 2014), suggested that hormetic dose responses might occur for other agents that affect pollen germination and/or pollen tube elongation. However, no integrative study has assessed the relevant literature for hormetic responses on pollen germination and tube elongation so far, prompting the present evaluation.

Hormesis is a biphasic dose–response relationship that is characterized by low-dose stimulation and high-dose inhibition (Cutler and Guedes, 2017). The magnitude of the low-dose stimulation is modest, with the maximum stimulation typically being in the 130–160% range (compared to control groups: 100%) (Calabrese and Blain, 2011). The dose width of the low-dose stimulation is usually less than a 50-fold starting from the estimated toxic/pharmacological threshold (Calabrese, 2008a, 2010, 2013). Hormetic responses may occur either by a direct stimulation or an overcompensation to a disruption in homeostasis and/or slight to modest toxicity. Preconditioning-mediated biological responses are examples of hormesis, displaying the typical hormetic dose response when sufficient conditioning doses are used in the experiment (Calabrese, 2016a,b). Hormetic dose responses quantitative characteristics display considerable generality in plants and other organisms, being independent of biological model, inducing agent,

endpoint, level of biological organization, and mechanism (Agathokleous et al. 2020a). This paper assesses the published literature on the occurrence of low doses/concentrations of chemical and physical agents on critical aspects of pollen biology, such as pollen germination and pollen tube elongation. Numerous examples of hormetic dose responses are presented and evaluated within historical and current biological settings as this is the first broadly integrated study to explore pollen biology within an hormetic framework. The paper will also relate the hormetic dose-response findings for pollen to the broader hormetic literature and highlight their potential implications for plant biology.

2. LITERATURE SEARCH STRATEGY & STATISTICS

PubMed, Web of Science, and Google Scholar databases were searched for articles using the terms hormesis or hormetic or biphasic dose-response or, U-shaped dose response or adaptive response, or preconditioning in combination with pollen germination or pollen elongation. All relevant articles were iteratively evaluated for references cited and for all papers citing these papers. All research groups publishing these articles were assessed for possible relevant publications in the above databases.

Twenty five papers with data of plants showing hormesis with pollen germination or pollen tube elongation were finally identified for inclusion in this study (Table 1). Data were collected from the original papers. Data were manually extracted from figures of the original papers. The response data were transformed into relative response, i.e. the maximum stimulatory response of a chemical treatment (at a non-zero dose) as a percentage of the response of control group (typically a zero dose) (Supplementary Materials).

To test if the maximum stimulatory response differed significantly between germination and tube elongation, the collated data set of each pollen trait was subjected to the nonparametric Mann–Whitney–Wilcoxon (MWW) test, after rejecting null hypothesis that the data are sampled from a normal distribution (chi-square goodness of fit test = 72.2, $P < 0.001$). Data were processed and analyzed with EXCEL 2010 (Microsoft Corporation) and STATISTICA v.10 (StatSoft Inc.).

3. HISTORICAL FRAMEWORK: EARLY HORMETIC FINDINGS

The initial report on the effects of chemical agents in a dose-response context on the germination and growth of pollen grains was made by Smith (1942) using the agent indole acetic acid (IAA) on snapdragon (*Antirrhinum majus*). The study of Smith (1942) made use of the sugar-agar technique which represented a significant improvement over the high drop technique that was commonly used in the 1920's and 1930's. With the sugar-agar technique the growth of pollen tubes was nearly double that compared with the earlier drop technique. Efforts were also made to select the optimal flower bud stages. Numerous other methods were adopted to minimize variation in the Smith (1942) report. In the IAA study, six concentrations were used ranging from 1/12,500 to 1/400,000 dilution. Low concentrations of IAA increased both germination and tube elongation (Smith, 1942) (Figure 1).

The 1942 findings of Smith were soon extended by Addicott (1943), who assessed 33 pure growth substances and several other agents for pollen germination and pollen tube growth in two species, the monocotyledon *Milla biflora* and the dicotyledon *Tropaeolum majus*. In using these two species it was recognized that pollen of both species required water, inorganic salts, a source of energy such as sucrose, and a mixture of various hormonal growth factors and other

nutrients. The media also included boron at the optimal concentration (0.01%), the first such study to include boron optimal dosing. Using the hanging drop method (that was rejected in the previous study by Smith, 1942), responses were reported over the range of concentrations of 0.01-100 mg/L. While temperature was shown in future studies to be important in affecting the hormetic dose response for pollen biology, the study by Addicott (1943) could not control temperature, using a room temperature of 26+/- 2^{o}C. In this large study of several dozen agents there was a broad spectrum of growth factors tested. The two plant species tested displayed some evidence of stimulation of pollen germination, pollen tube elongation, or both. Under such broad testing, it is expected that some of these responses would have occur by chance alone. However, hormetic dose-response patterns as represented by a low-dose stimulation and a high-dose inhibition were commonly observed for pollen tube elongation with the *M. biflora* pollen (Figures 2 and 3). The maximum hormetic stimulation was shown to markedly vary across the agents differing up to 1000 fold (Figure 2). The hormetic dose-response phenomenon also occurred with multiple agents in the dicotyledon *T. majus* but was less consistently observed in this species as compared to *M. biflora*. In general, neither pollen model displayed hormesis for pollen germination, except for alpha napthyl acetamide, 2-chloroisothiamin-iodide, and guanine with *M. biflora*.

In the publication of Addicott (1943), there was an experiment which assessed the combination of seven agents for pollen germination and pollen tube elongation in both plant species. Neither endpoint in the two species showed evidence of synergy or additivity. The mixture response was 147% for pollen tube elongation for *M.* biflora, whereas the median response for these same agents when tested separately at the same concentrations tested was not significantly different (i.e. 142%). Comparable mixture studies with the 11 agents for *T. majus*

yielded similar findings with no evidence of synergy or additivity when compared to the group of separately tested agents using the same concentrations. These findings are important because they showed that responses greater than the experimentally-derived hormetic maximum were not observed in either model for the endpoints measured when mixtures were compared summed responses of the same individual agents.

Several follow up studies provided data for multiple agents over a relatively broad dose/concentration range (Raghavan and Baruah, 1956a,b, 1959; Vasil, 1960; Kwan et al., 1969; Bamzai and Randhawa, 1967). These studies tended to use some of the same agents but employing a different plant species while generally following similar methods. In the cases of Raghaven and Baruah (1956a,b, 1959) and Vasil (1960) neither temperature nor humidity were controlled. Hormetic-biphasic dose responses were reported for boron compounds, galactose, IAA, succinic acid, fumaric acid (Vasil, 1960), and a bulb extract mixture (Vasil, 1960; Kwan et al., 1969; Raghavan and Baruah, 1959). In contrast to the findings of Addicott (1943), there was an hormetic dose response for both germination and pollen tube elongation in these experiments.

4. RADIATION - UV AND IONIZING

In the June, 1971 issue of the Stimulation Newsletter, Zelles et al. (1971) made the first attempt to assess the effects of a wide range of UV radiation on the pollen germination rate and pollen tube length using *Pinus sylvestris*. This research was stimulated by debates in the literature that low doses of ionizing radiation induce opposite responses to those produced at higher doses (i.e. see historical foundations of chemical and radiation hormesis - Calabrese and Baldwin 2000a-e). Zelles et al. (1971) noted that several recent studies had supported this

perspective and encouraged their mechanistic follow up research. They selected the use of UV-radiation since its molecular toxicity mechanisms were reasonably known. In their study, pollen was exposed to UV-radiation ranging from 0.3-144.0 $X10^5$ erg/cm^2. The data indicated an hormetic-like biphasic dose response for pollen germination. The pollen tubes were also elongated at the dose maximally enhancing germination (2.4 X 10^5 erg/cm^2) (Figure 4; Zelles et al., 1971). The elongation was blocked by antinomycin during specific phases of germination, revealing that elongation stimulation was dependent on RNA synthesis. Follow up experiments by Zelles and Ernst (1972) confirmed the hormetic biphasic dose response assessing pollen tube elongation over the UV range of 1.2-70 x 10^5 erg/cm^2, both with respect to the summed lengths of the pollen tubes but also the number of tubes > 150 um in length.

Since the previous research of Zelles et al. (1972) estimated that the growth of pollen tubes can be enhanced by UV-irradiation, Fendrikc and Zekes (1971) sought to determine whether X-rays or gamma radiation could also similarly affect pollen tube growth in *P. sylvestris*. Using X-rays with two different energies (30 and 300 kV) with the dose rate of 60 R/min, X-ray treatments enhanced pollen tube growth at 300 R with biphasic dose responses in the range of 30-1000 r (or 3000 r). The maximum stimulation was in the 140-150% range for both treatments. A similar biphasic dose-response pattern was shown for Co-60 with the peak occurring at 1000 r, with the maximum response at 127%.

The impact of dose-rate was more fully investigated in later studies by Zelles and Fendrik (1975). From 0.5 to 5.0 rad/sec significant elongation was observed, whereas above 10 rad/sec inhibition occurred. More detailed follow up experiments revealed the low-dose stimulation to be reproducible, but highly dependent on dose rate. The important influence of UV dose rate and exposure duration were shown in a follow up study by Seibold et al. (1979). However, regardless

of the variation in experimental protocols, the maximum stimulation and stimulatory dose range were very consistent. In both the UV and X-ray studies the stimulatory effects started to occur at less than 25% of the LD50 value, a response consistently reported in the hormesis literature (Calabrese and Blain, 2011; Nascarella and Calabrese, 2009). The collective observations by Zelles and colleagues led to the conclusion that "when irradiation was carried out slowly the effect of stimulus of dose response is reproducible and statistically significant." The stimulatory response of low-dose rates was seen as an overcompensation repair process that is not observable at higher doses.

5. POLLUTION AND POLLEN

The effects of environmental pollutants on pollen have been extensively investigated. Toxic substances in the environment can affect pollen germination and tube elongation. While often assessed within the context of adverse effects, in some plant species low atmospheric levels of halogens (Konishi and Miyamoto, 1983; Portyenko and Kudrja, 1966), heavy metals (de Bruyn, 1966; Jolub and Ostroluska, 1983; Searcy and Mulcahy, 1985), automobile exhaust (Fluckinger and Braum, 1977), simulated acid rain (Cox, 1983; Masaru et al., 1980), and metallic salts (e.g. Mn $(NO_3)_2$, Pb $(NO_3)_2$, HNO_3, HCl, and H_2SO_4) enhance pollen germination and pollen tube growth. Further, several groups also noted pollen germination and tube elongation at concentrations up to 1000 ppm of ethylene (Buchannen and Briggs, 1969; Search and Stanely, 1968). Of particular significance in the assessment of pollutant effects on pollen biology have been studies with various metallic elements, including cadmium. In one particular study, Xiong and Peng (2001) assessed both pollen germination and pollen tube elongation in five plant species across seven concentrations ranging from 0.00001 to 6.30 μg/ml (Figures 5A,B). While

there was considerable interspecies variation with respect to the optimal (i.e. stimulatory) concentration, each species displayed hormetic biphasic dose responses for pollen tube elongation (Figure 5B). The optimal concentrations ranged from 0.0001 ug/ml to 0.1 ug/ml, a factor of 1000-fold. In fact, while one species was showing its optimal response (184.2 %; *P. degrassa*) another species was inhibited by 36.8% (B. *tetrasperm*a). While all species displayed hormesis for tube elongation, hormetic responses were not observed in any species tested for germination (Figure 5A).

A similar enhanced capacity for pollen tube hormetic stimulation was reported by Tuna et al. (2002) in tobacco plants. In the case of $FeCl_2$ suggestive hormetic findings also occurred for germination. The study by Searcy and Mulcahy (1985) indicated that copper induced an hormetic response for germination and pollen tube elongation in copper tolerant sporophytes, while the stimulation did not occur in those individuals lacking the induced tolerance (Figure 6). Thus, pollen from tolerant individuals displayed not only acquired resilience but also the capacity to display enhanced germination and pollen tube elongation at concentrations of copper which adversely affected the non-tolerant individuals. Finally, in a study of ozone and its related peroxides on pollen, short-term exposures stimulated pollen tube elongation with both hydrogen peroxide and tert-butylhydroperoxide (10^{-8} to 10^{-4} M) (Figure 7). Roshchina and Mel'Nikova (2001), who noted that peroxide–induced activation of seed germination was well known, concluded that at low concentrations such products of ozone exposure do not adversely affect pollen grains but enhance pollen germination: "It is not inconceivable that low ozone dosage may turn out to be beneficial since ozone and peroxides produced upon ozonolysis interact with the pollen surface not only as oxidants, but also as chemical signals."

6. POLYAMINES

An area of research on the effects of chemicals on pollen germination and pollen tube elongation involves polyamines (Cetinbas-Genc, 2020; Cetinbas-Genc et al., 2020). The first suggestion of such an involvement was published by Bagni et al. (1981) who showed that synthesis of polyamines occurs in apple pollen during germination. In the first dose-response follow-up investigation, Prakash et al. (1988) reported that the polyamine spermidine induced a biphasic dose response (*in vitro*) in *Catharnathus roseus* for pollen tube length. Blockage of protein synthesis prevented the stimulatory response. A decade later Song et al. (1999) extended these initial findings with research on tomatoes showing that spermine induced a similar biphasic dose response for both germination and pollen tube length at 25°C or 33°C. The effects of the spermidine (Figure 8) and spermine were greater at 33°C as compared to 25°C. Further, the stimulatory response was comparable for the germination and pollen tube elongation at both temperatures. These findings were extended by Wolukau et al. (2004) who showed that both temperature and polyamines affected the germination and pollen tube elongation in *P. mume*. These parameters were stimulated to a greater extent at 10°C as compared to 25°C.

A similar follow up study by Sorkheh et al. (2011) with a different cultivar of *P. mume* showed similar findings with hormetic-like biphasic dose responses for both germination and tube elongation occurring at 10°C while to a lesser extent at 25°C. These biphasic dose-response findings were followed with preliminary mechanistic information. At the higher inhibitory doses putrescine was reported to affect the occurrence of excessive accumulation of reactive oxygen species, adversely affecting pollen tube functions (You and Chan, 2015; Cetinbas-Genc, et al., 2020). A recent report by Cetibas-Genc, 2020 indicated that putrescine enhancement of pollen tube elongation was associated with alterations and actin filaments in the apex, while at higher

doses this trend was reversed. These findings indicate that within the hormetic range the actin filaments became more dynamic for the transport of materials required for tube elongation while losing this capacity at higher concentrations. At lower stimulatory doses there was an increase in several antioxidant enzyme activities, including superoxide dismutase and catalase.

7. DISCUSSION

There has been a prolonged interest in chemical and physical agents that could affect pollen germination and pollen tube elongation given their central role in plant biology and reproductive success. While the principal focus has been on efforts that optimize or enhance pollen germination and/or pollen tube elongation, concerns have also been directed toward identifying and assessing agents that may adversely affect these critical aspects of pollen biology. These studies have spanned nearly eight decades, including a broad range of agents, such as nutrients, numerous growth promoting agents, products of endogenous metabolism, hormones, toxic metals, air pollutants, components of acid precipitation as well as ionizing and non-ionizing radiation. Evidence shows that agents in each of these diverse categories enhanced pollen germination and/or pollen tube elongation of various species (Table 1) in a manner consistent with the quantitative features of the hormetic dose response (Figures 1-8 and S1-S25).

The maximum stimulation for germination across all studies (N = 45) was 142.0% (median) (166.1%-mean) while the width of the stimulation range was 5-fold (median). This matched very closely with results of the tube elongation studies (N = 70) reviewed here, which had a 147.8% median maximum stimulation (171.9%-mean) along with a 5 fold median stimulatory dose/concentration width. According to MWW test results, the distributions of both populations (Figure S27) are equal (U = 1398, Z adjusted = 1.01, P = 0.312). This analysis is in

agreement with the only previous analysis of the magnitude of the maximum stimulatory response among specific endpoints in plants, which revealed no significant difference among photosynthetic pigments (Agathokleous et al., 2020b). These analyses support the hypothesis that the quantitative features of hormesis are independent of response endpoints.

Despite the long period of dose/concentration research on germination and tube elongation and the relatively large number of agents inducing hormetic dose responses, there has been relatively little focus on underling mechanisms, especially as compared to many other biological/biomedical areas exploring hormetic process. This is particularly interesting since the area of pollen biology has not exploited experimental biological models sufficiently within the context of detailed mechanistic understanding as compared to many other areas (e.g. cell proliferation and numerous chemoprotective endpoints). This may be related to the fact that numerous examples of hormetic responses with pollen occurred between 1940-1980, prior to the onset of modern cell signaling developments. Furthermore, areas of pollen biology that were evaluated prior to the 1980's did not generate substantial mechanistic follow up research. For example, several of the radiation pollen papers of Zelles were published in the *Stimulation Newsletter* (1970-1975), a publication that was short lived and not indexed. Follow up publications in well-known journals on this topic by Zelles received very few citations (i.e. less than 15) in the Web of Science over the following 50-year period, despite being well designed, executed, and with good reproducibility.

The findings of Searcy and Mulcahy (1985) that metal-tolerant plants display hormetic stimulation represents a unique type of preconditioning (Calabrese, 2016a,b) experimental protocol with strong application to a large number of possible environmental scenarios.

However, as with the case of Zelles for ionizing radiation, inadequate follow-up research occurred.

The demonstration by Addicott (1943) that multiple hormetic-acting agents when tested together at optimal hormetic doses did not result in an increased response beyond that of the most stimulatory single compound is similar to result of other studies with complex mixtures (e.g. memory enhancing drugs: (Calabrese, 2008b), waste water effluent (Calabrese, 2008a), plant extract material such as ginseng (Calabrese, 2020). These observations support the hypothesis that the maximal hormetic stimulation defines a type of biological plasticity. These findings indicate that the concept of synergy within an hormetic stimulation differs from what has been typically studied within toxicological evaluations in which the toxicity increases. Synergy within an hormetic concept occurs within the response framework up to the limits of biological plasticity (Calabrese, 2008c; Agathokleous et al., 2020a). This differentiation of the synergy concept within these two biological contexts is essential to note since agents that induce enhancement of biological performance, rather than toxicity, will be limited to increase the hormetic maxima.

The relationship of germination and pollen tube elongation remains to be better clarified. Many studies cited here report hormetic dose-response relationships for both endpoints. However, there was no generally consistent association of these hormetic responses in the same experiments. In some studies, the hormesis stimulation occurred for both parameters but there were numerous exceptions where this was not the case. In other cases, in which both parameters displayed hormetic responses, the optimal concentrations for these endpoints were considerably different. Regardless of the lack of generally correlated hormetic responses in the same

biological model, both germination and tube elongation parameters often displayed hormetic responses with similar quantitative features.

In addition to there being a wide range of chemical and physical agents inducing hormesis in pollen, the range of plant species selected for study and displaying hormetic responses was also extensive. A review of all papers inducing pollen hormesis revealed many different factors that affected the selection of the plant species. Some of these reasons included the economic significance of the plant species, past experience with the biological model, and the reliability/reproducibility of findings, amongst others. However, the widespread occurrence of hormesis in such a diverse setting of plant pollen supports the generality of the hormesis concept.

The hormetic dose-response findings for pollen germination and pollen tube elongation can be viewed within the framework of the more general context of overall plant hormetic dose-response relationships (Calabrese and Blain, 2009, 2011; Muszyńska and Labudda, 2019; Carvalho et al., 2020; Agathokleous et al., 2020a) that display hormetic dose responses in both direct stimulatory and preconditioning experimental protocols. The present assessment further generalized the concept of hormesis, which had not been a previous focus for pollen biology within an integrated dose/concentration context. Recognition that the hormetic dose response commonly occurs in pollen biology, at least with respect to germination and tube elongation, has the potential to be of theoretical and practical utility of researchers in this area with respect study design, dose selection and dose spacing.

Because pollen male reproductive success requires an expeditious and successful tube growth, the development of pollen and the growth of pollen tubes require high energy (Selinski and Scheibe, 2014). The herein extensive documentation of stimulation of pollen germination and pollen tube elongation by low doses of many stresses, including various pollutants occurring

in the environment, suggests that stimulated pollen development may imply readjustments of underpinning mechanisms of balancing energy, such as mitochondrial respiration and fermentation, plastidial glycolysis, and the ATP/NAD(P)H ratio (Selinski and Scheibe, 2014). Hence, new studies are needed to address the effects of such physiological changes on the fitness of individual organisms, occurring at doses of stress that are considerably below the traditional toxicological threshold. During the critical stage of fast pollen development, increased investments of energy resources to pollen germination and tube elongation might be costly in terms of plant defense, thus having potential unpredicted consequences to the interaction of plants with pests and other infectious or non-infectious biological organisms. Hence, at this stage, it cannot be concluded that the stimulation of pollen germination and pollen tube elongation by low-dose stress is beneficial to the plant. Further studies are needed to evaluate potential effects of low-dose stimulation of pollen germination and tube elongation on plant defense and inter- and intra-specific biotic interactions, as low-dose stimulation displays also considerable within-population variability (Agathokleous et al., 2020a).

Responses of pollen that are indicative of hormesis, have been induced by nutrients, growth-promoting agents, plant and animal hormones, and toxicants, including heavy metals, gaseous pollutants, and ionizing and non-ionizing radiation. The wide occurrence of such stresses from local to global scales (Larsson, 2014; Nagajyoti et al., 2010; Sicard et al., 2017, 2020; Thomas and Symonds, 2016) suggests that agricultural and ecological implications should be of global concern, especially because lower doses (below the toxicological threshold) are more likely to occur than high doses exceeding the toxicological threshold.

Enhanced pollen germination and lengthened pollen tube in the presence of low-dose stresses might enhance the chances for successful pollination and in general enhance pollination.

In turn, enhanced pollination may favor the quality of the offspring (Holm, 1994). This is not only ecologically important for maximizing the chances for the species survival, but also agriculturally important for producing improved offsprings and facilitating the pollination in crop cultures of which success depends on pollination, such as tomato that often depends on bumblebee-mediated pollination (Banda and Paxton, 1991). Nonetheless, the agricultural implications of such hormetic responses of plants to low-dose stresses, reflected to pollen biology, should be further explored.

A question of high ecological interest is whether the stimulatory response of pollen to low doses of stresses differs between angiosperms and gymnosperms. A key characteristic of angiosperms is the rapid growth rate of pollen tube (Williams, 2008), and it has been suggested that pollen germination and tube growth rates should be faster in extant angiosperms than other spermatophytes (Williams, 2012b). However, it remains unclear how and why rapid growth rates evolved in angiosperms (compared to conifers and Gnetales) and why the rates of pollen tube growth highly vary within angiosperms (Williams et al., 2016). Conifers differ from angiosperms in that their pollen tube rate of growth is considerably slower, their pollen tube growth period is extended, their sperm formation is considerably delayed, and no cytokinesis follows the formation of sperm (Fernando et al., 2005). The pollen tube wall of conifers is also predominantly composed of cellulose, and distinct cytoskeletal control and organelle zonation (Fernando et al., 2005). Hence, there are considerable differences in the pollen biology between conifers and angiosperms, raising the question of whether these are reflected to the magnitude of the stimulatory response of pollen to low doses of stresses. From 30 taxa identified to show such biphasic dose responses, 28 were angiosperms and only 2 gymnosperms (Table 1). Likewise, from the 28 angiosperms, 23 were eudicots and only 5 were monocots (Table 1). Therefore, the

limited sample size for gymnosperms and monocots did not permit robust comparisons of the maximum stimulatory response among functional groups. Although pollen tubes of conifers represent a key evolutionary step in the development of male gametophyte, they have been underexplored in plant biology (Fernando et al., 2005). Pollen tubes of conifers are an intermediate form between the haustorium-type pollen tubes of *Ginkgo* or cycads and the structurally simplified and faster-growing pollen tubes of angiosperms (Fernando et al., 2005). Further studies on the effects of low doses of environmental stresses on pollen tube growth of conifers are needed to understand whether low-dose responses of pollen differ between conifers and angiosperms.

Pollen germination and tube growth precede seed set and are critical for successful fertilization (van Tussenbroek et al., 2016). The primary process influenced by pollen competition among early angiosperms might be pollen germination (Williams, 2012b). Both pollen germination and tube growth are important components of reproductive biology and major aspects of the evolution of plants (Williams, 2009, 2012a), and the interaction between the gametophyte and the flower sporophytic tissues may have implications to plant diversity and evolution (Lora et al., 2016). The processes of pollen germination and pollen tube growth may exhibit some modularity and evolve at different rates in situations where there is a difference in the maternal control over the form and/or intensity of competition between the stigma and the stylar canal or ovary (Williams, 2012b). However, the knowledge about the evolutionary developmental relationship between germination speed and pollen tube growth rates is limited (Williams, 2012b).

The pollen tube growth economics depend on tube design, as a result of trade-offs between efficient growth of pollen tube and other functions of pollen tube (Williams et al.,

2016). The performance of male gametophyte also depends on the rate of pollen tube elongation, because the synchrony and duration of the fertilization process constrains growth rate, often in the presence of competition among pollen tubes for access to eggs (Williams et al., 2016). If low doses of stresses lengthen the pollen tube, it may be postulated that less time is needed from the style up to the embryo sacs. Why should plants spend so much energy to fasten the time needed for pollen tubes? This becomes even more fascinating for angiosperms, which have anyway evolved a rapid pollen tube growth (Williams et al., 2016). One may hypothesize that this phenomenon might be the outcome of a rescue strategy where a species (or individual) senses forthcoming threats and expedites the fertilization and reproductive process so to produce seeds as soon as possible. The ecological implications of a potentially expedited fertilization and reproductive process may have implications to not only male-female interactions but also male-male competition depending on the individual sensitivity to environmental stresses, considering the within-population variability of low-dose sensitivity (Agathokleous et al., 2020a). Furthermore, pollen tube pathways are diverse within spermatophytes (Lora et al., 2016), but how the architecture may also change along with elongation under the influence of low-dose stress remains unknown.

The biological mechanisms explaining the need for enhanced pollen tube elongation by low doses of stress remain unknown too. Calcium ion (Ca^{2+}) is critical in the control of pollen germination and tube growth (Zheng et al., 2019), although there are differences between conifers and angiosperms (Fernando et al., 2005). Ca^{2+} is involved in stress signaling and the regulation of ion homeostasis (Manishankar et al., 2018), being affected by oxidative stress (Greene et al., 2002). Ca^{2+} can protect plants against stress (Zhao and Tan, 2005), and its mitochondrial and cytoplasmic levels can increase in response to stress (Greene et al., 2002).

How Ca^{2+} might be linked to the hormetic responses of plants reflected to pollen germination and growth elongation should be tested in new studies. It is also important to examine whether the stimulatory response of pollen tube growth is primarily associated with increased lipids at the surface of the stigmata.

8. CONCLUSION

This is the first study evaluating the literature of pollen development as affected by various abiotic factors in the context of hormesis. The herein assessment revealed ample evidence of hormetic responses of pollen germination and tube elongation to various pollutants and other agents in many plant species, with quantitative features that conform with the general understandings of hormesis. New research agendas should set forth to investigate the potential implications of stimulation of pollen germination and tube elongation by low doses of abiotic stresses to plant defense, fitness, and interactions with other organisms.

9. ACKNOWLEDGEMENT

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involvement in study design, collection, analysis, interpretation, writing and decision to and where to submit for publication consideration.

10. CONFLICT OF INTEREST

Authors declare no conflict of interest

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Table 1. Plants showing hormesis with pollen germination or pollen tube elongation. Dose/concentration-response relationships are presented in the Supplementary Materials. IAA= Indole-3-Acetic Acid; PABA = Para-Aminobenzoic Acid; IPA = 3-Indole-Proprionic Acid; MHA = 2-Methyl-4-Hydroxy-5-Aminomethylpurine-hydrochloride; NPA = 1-N-Naphthylphthalamic Acid; GA = Gibberellic Acid

Taxon	**Functional Group**	**Agent Inducing Hormesis**	**Reference**
Allium sativum	Angiosperm (monocot)	IAA, Succinic acid fumaric acid	Kwan et al 1969
Antirrhinum majus	Angiosperm (eudicot)	IAA	Smith, 1942
Arabidopsis thaliana	Angiosperm (eudicot)	Epibrassinolide	Vogler et al., 2014
Areca catechu	Angiosperm (monocot)	PABA, IPA, IAA, $MnSO_4$, $CoCl_2$, Boron	Ragavan and Baruah, 1959
Betula nigra	Angiosperm (eudicot)	Radiation	Clausen, 1977
Calotropis gigantea	Angiosperm (eudicot)	Maize seed extract	Viswanathan and Lakshmanan, 1984
Camillia sinensis	Angiosperm (eudicot)	Rapeseed cakes	Konishi and Yokotai, 1980
Cassia obstusifolia	Angiosperm (eudicot)	Sucrose	Yadav, 1980
Catharantus roseus	Angiosperm (eudicot)	Spermidine	Prakash et al 1988
Corylus avellana	Angiosperm (eudicot)	Putescine	Çetinbas-Genç et al., 2020
Cucumis melo	Angiosperm (eudicot)	Boron	Vasil, 1960
Glycine max	Angiosperm (eudicot)	Aflatoxin B	Jones 1980
Hippeastrum hybridum	Angiosperm (monocot)	Hydrogen peroxide and tert-butylhydroperoxide	Roshchina and Mel'nikova, 2001
Lycopersicon esculentum	Angiosperm (eudicot)	Hydroperoxide	Song et al, 1999

Taxon	Functional Group	Agent Inducing Hormesis	Reference
Medicago hispida	Angiosperm (eudicot)	Cadmium	Xiong and Peng, 2001
Milla biflora	Angiosperm (monocot)	thiamine, niacin, IAA, uric acid, MHA, alloxon, PABA, pyridoxine, alpha napthyl acetamide, traumatic acid	Addicott, 1943
Mimulus guttatus	Angiosperm (eudicot)	Copper	Searcy and Mulcahy, 1985
Pinus silvestris	Gymnosperm (conifer)	UV radiation	Zelles, 1971
Prunus mume	Angiosperm (eudicot)	Spermidine	Wolukau et al., 2004
Picea meyeri	Gymnosperm (conifer)	Boron	Wang et al., 2003
Pisum sativum	Angiosperm (eudicot)	Cadmium	Xiong and Peng, 2001
Plantago depressa	Angiosperm (eudicot)	Cadmium	Xiong and Peng, 2001
Prunus duclis	Angiosperm (eudicot)	Spermidine	Sorkheh et al., 2011
Setaria sphacelata	Angiosperm (monocot)	Thymindine, ascorbic acid, nicotinic acid, and pyridoxine	de Bruyn, 1961
Nicotiana tabacum	Angiosperm (eudicot)	$FeCl_2$	Tuna et al, 2002
Torenia fournieri	Angiosperm (eudicot)	NPA	Wu et al, 1988
Tropaeolum majus	Angiosperm (eudicot)	(tropaolum)-2-methyl-4-amino-amino-methylpurine-hydrochloride	Addicott, 1943

Taxon	Functional Group	Agent Inducing Hormesis	Reference
Vicia sativa subsp. *nigra*	Angiosperm (eudicot)	Cadmium	Xiong and Peng, 2001
Vicia tetrasperma	Angiosperm (eudicot)	Cadmium	Xiong and Peng, 2001
Vitis vinifera three cultures (Pearl Csaba, Pusa Seedless, and Bhokril) and two hybrids (Bagalore Blue and Golden Queen)	Angiosperm (eudicot)	GA, IPA, IAA, $MNSO_4$, $CoCl_2$, boron	Bamzai and Radhawa, 1967

Figure 1. Effects of 3-indole acetic acid on the tube length of snapdragon pollen (Data: Smith, 1942)

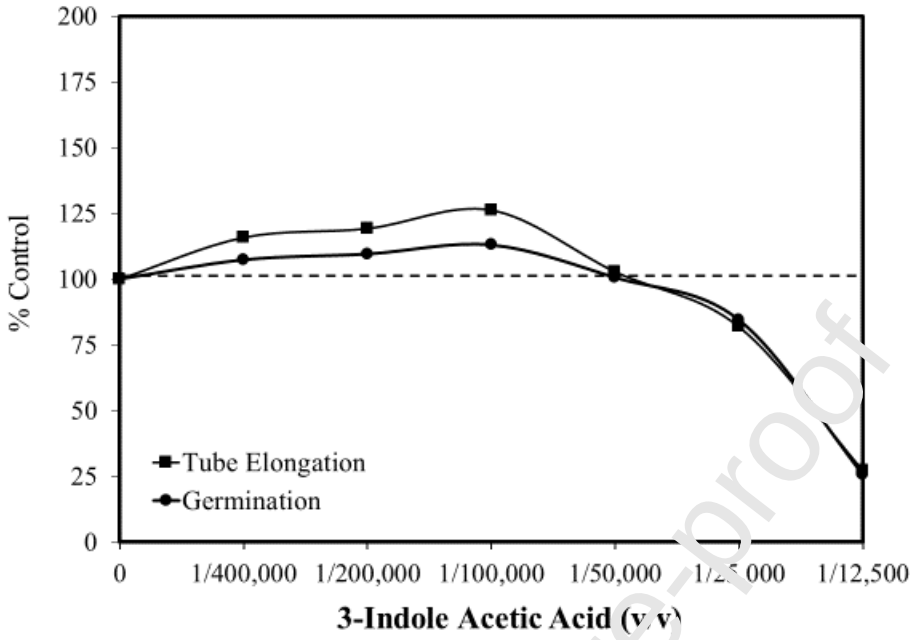


Figure 2. Effects of pure growth factors on tube growth of *Milla biflora* pollen (Data: Addicott, 1943)

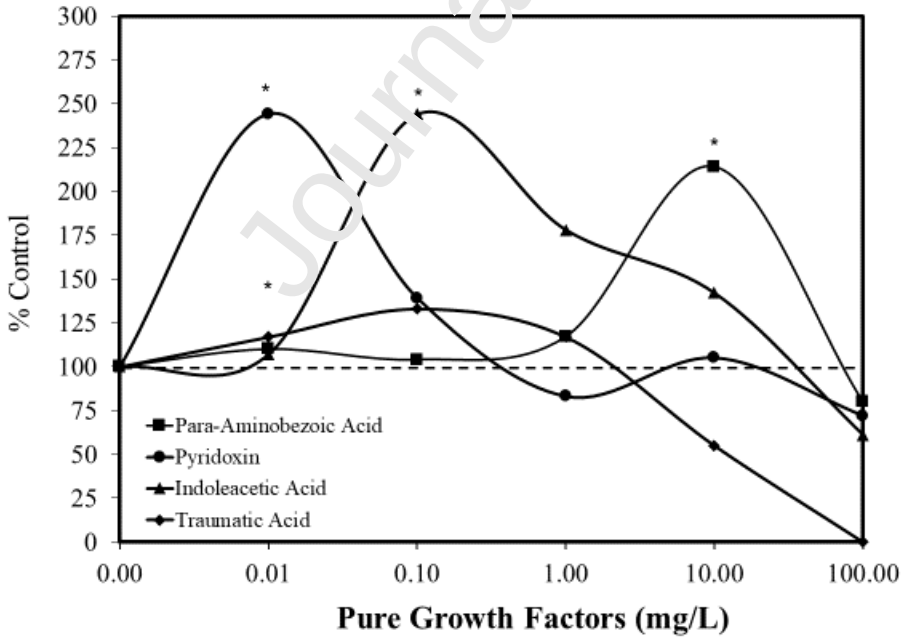


Figure 3. Effects of pure growth factors on tube growth of *Milla biflora* pollen (Data: Addicott, 1943)

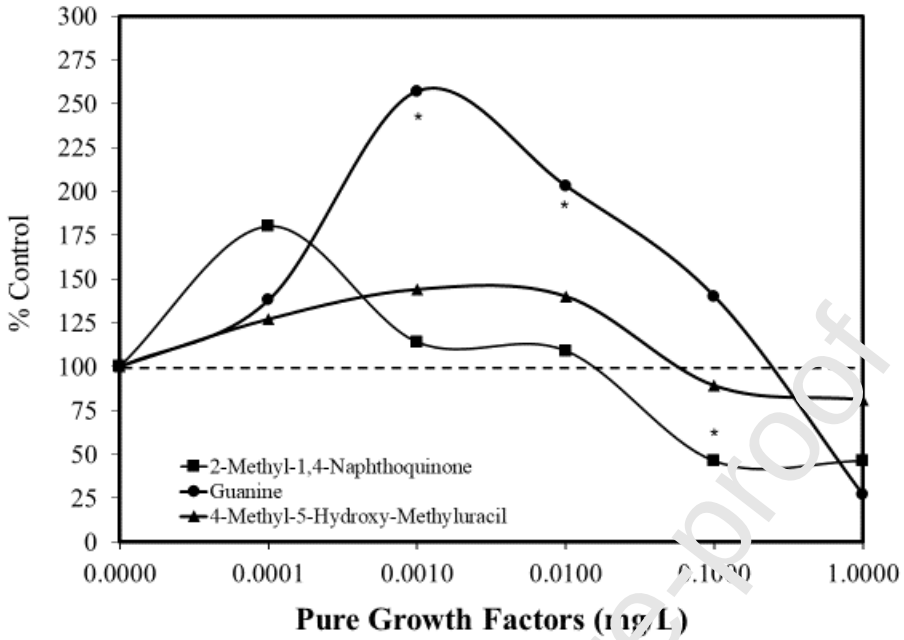


Figure 4. Effects of incident UV-fluence x 10^3 erg/cm^2 on pollen tube elongation of *Pinus sylvestris* (Data: Zelles and Ernst, 1972)

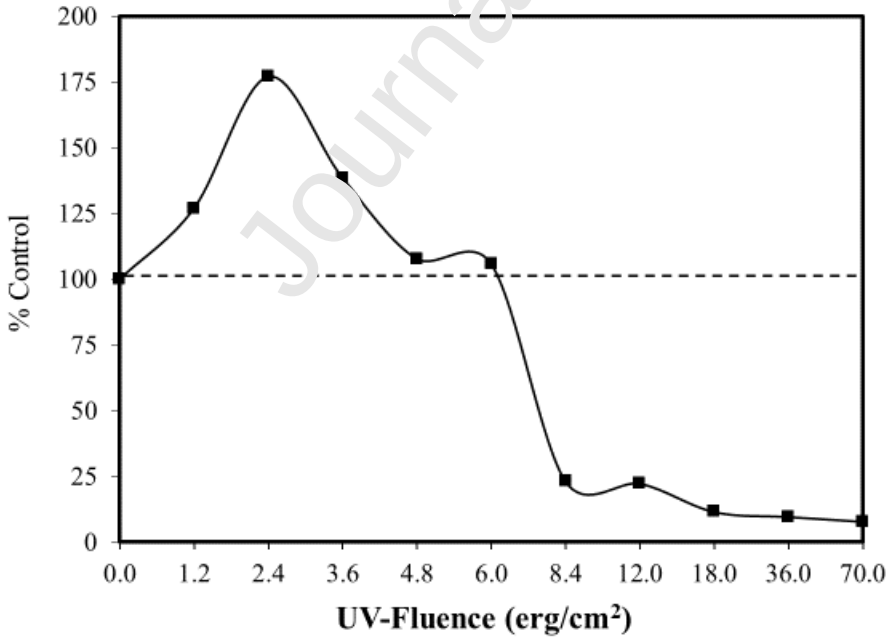


Figure 5A. Effects of cadmium on the relative pollen germination rates of five species exposed *in vitro* (Data: Xiong and Peng, 2001)

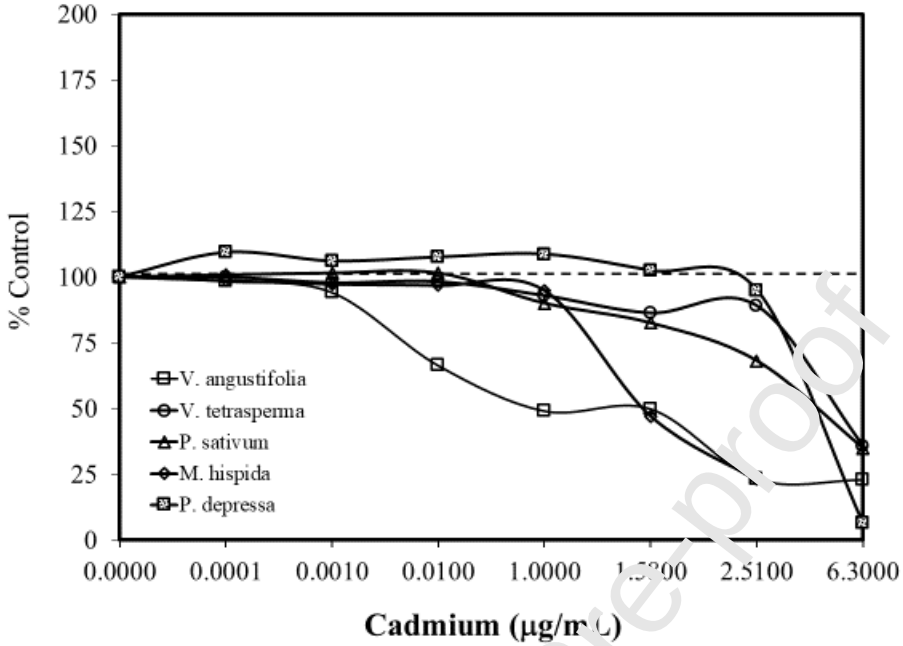


Figure 5B. Effects of cadmium on the relative pollen tube length of five species exposed *in vitro* (Data: Xiong and Peng, 2001)

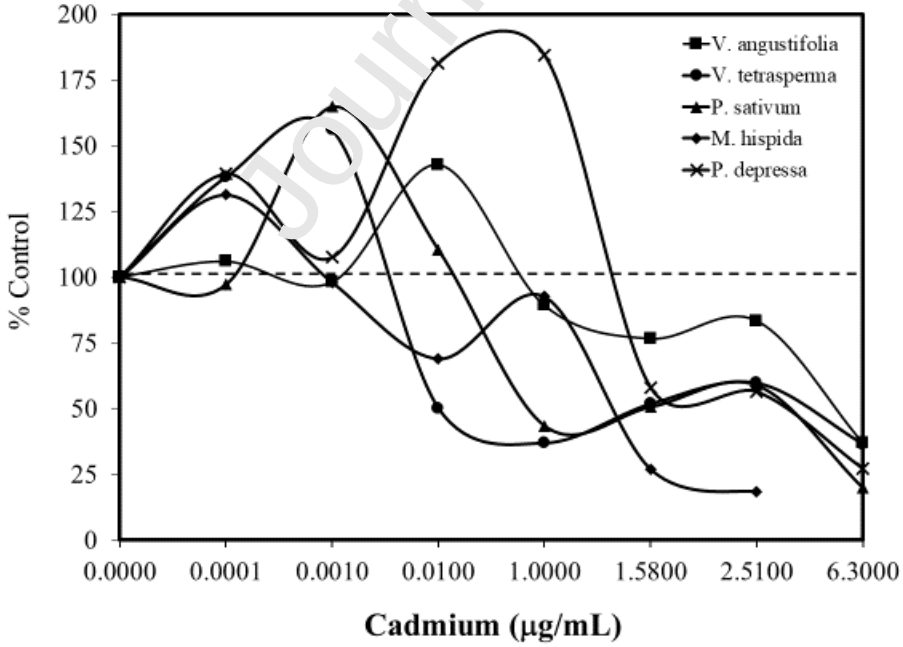


Figure 6. Effects of copper concentrations on percent germination and tube length of pollen from tolerant (M22-1) clones of *M. guttatus* (Data: Searchy and Mulcahy, 1985)

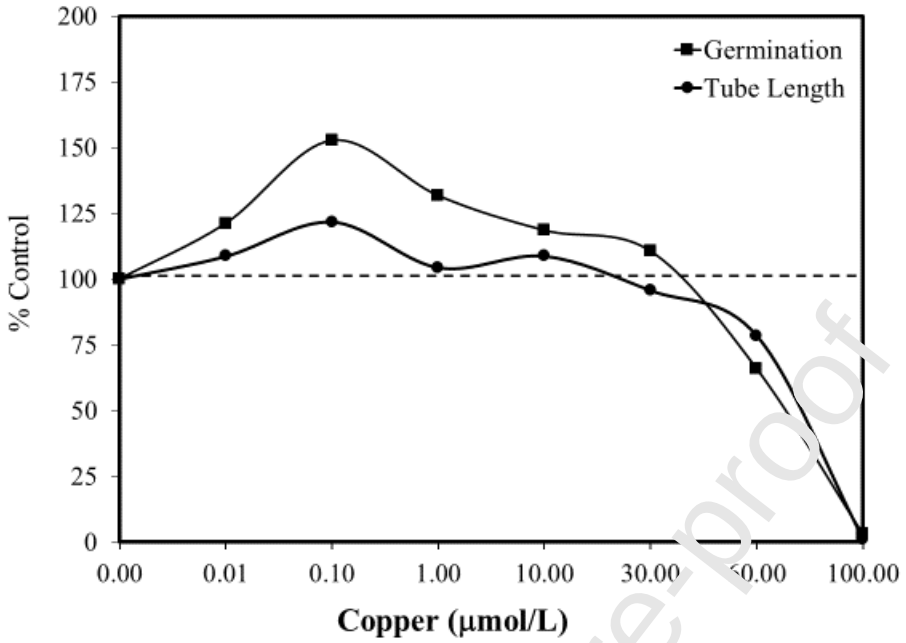


Figure 7. Effects of hydrogen peroxide and *tert*-burylhydroperoxide on germination of *Hippeastrum hybridum* pollen (Data: Reshchina and Mel'nikova, 2001)

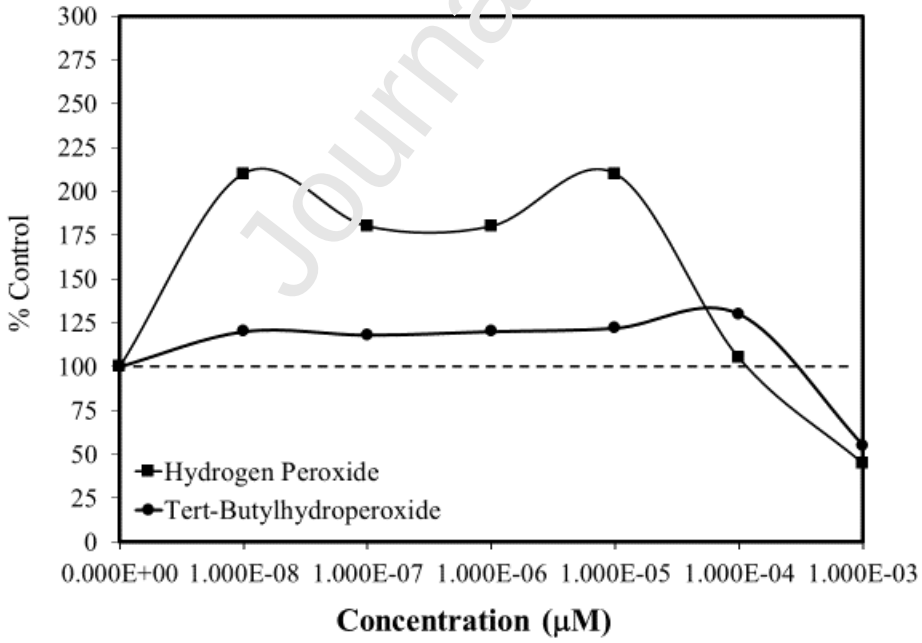
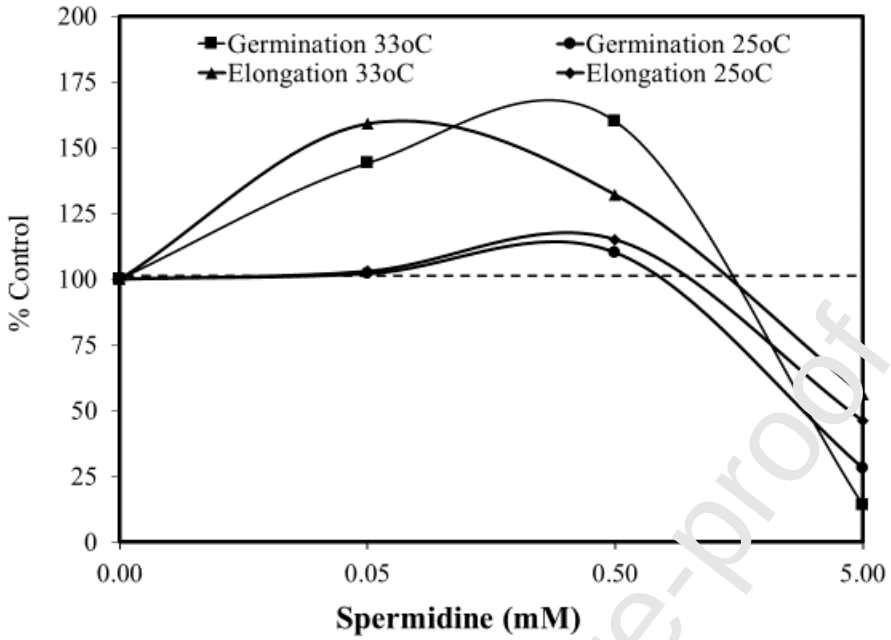


Figure 8. Effects of spermidine on the pollen germination and pollen tube elongation of a tomato (*Solanum lycopersicum* Jifen No. 3) (Data: Song et al., 1999)



Credit Author Statement:

EJCalabrese: conceptualization, formal analysis, funding acquisition, writing original draft, review and editing. EAgathokleous: review and editing.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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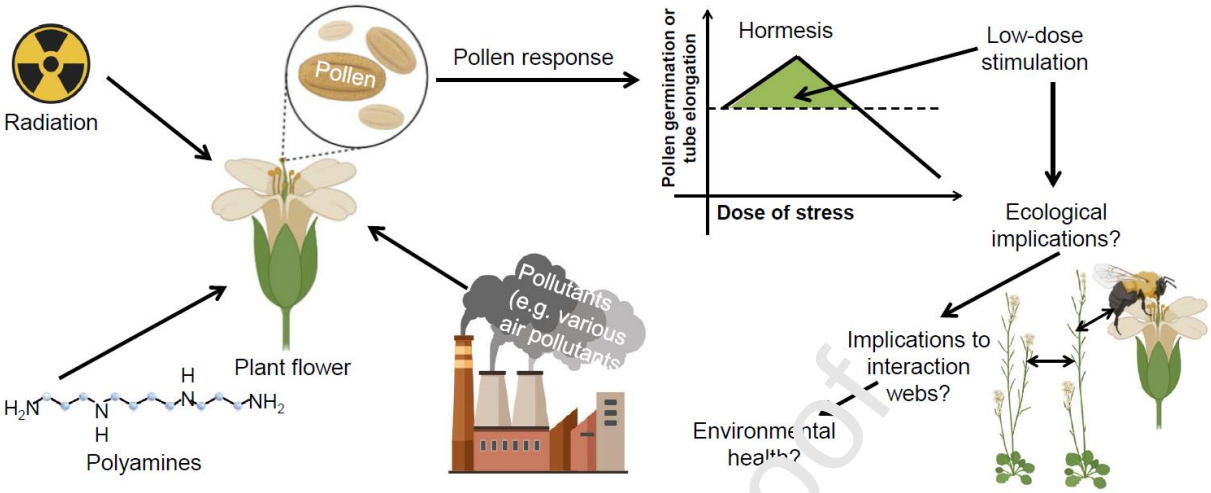
Graphical abstarct



- We collated ample evidence of hormesis in pollen germination and tube elongation.
- Hormesis was induced by various stresses, e.g. radiation, polyamines and air pollutants.
- The maximum stimulation was similar for pollen germination and tube elongation.
- The maximum low-dose stimulation was consistent with the broad hormesis literature.
- Low-dose responses of pollen endpoints may have unpredicted ecological implications.